Effects of Senescence on Vascular Smooth Muscle in the Yellow Mud Turtle *Kinosternon flavescens* (Family *Kinosternidae*)

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An abstract of a thesis by

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Current literature suggests that reptiles do not undergo senescence and certainly do not age in a manner similar to mammals. This study examines age-related changes in reptilian arteries to evaluate similarities to age-related changes in mammals. Arterial sections from a wild population of turtles ranging in age from 6 to 35+ years were examined histologically. Connective tissue-stained arteries showed statistically significant deposition of connective tissue in the tunica media with advancing age, consistent with senescent mammalian arteries. Living rings of arterial tissue from the same turtles were stimulated with potassium chloride, phenylephrine, acetylcholine, and isoproterenol to identify physiological responses in the vascular smooth muscle. No statistically significant changes in response related to aging were observed. Contractions were produced by acetylcholine, a feature typical of *Pseudemys scripta* and not of mammals. Isoproterenol did not produce contractions suggesting *K. flavescens* lack beta-2 receptors or these receptors do not operate similarly to mammals. The lack of decrease in arterial responsiveness in senescent arteries suggests that turtles may possess a mechanism for increasing myofibril strength in the presence of decreased myofibril mass and increased connective tissue.

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Introduction and Review of the Literature

Senescence is the deterioration that results from advanced age. Pathologies associated with the senescent process in mammals, especially humans, are well documented (Pathy, 1985). Senescence in reptiles is less well understood (Comfort, 1979) and it is postulated that reptiles do not undergo a senescence process (Gibbons, 1987, 1990). A reason for reptile longevity may be that egg mortality can be 95-99% (Christiansen and Gallaway, 1984) and that more reproductive years are required to insure the survival of a single clutch of eggs for any one female. A few studies have suggested some evidence of senescence in reptiles. Age-related decreases in stamina of lizards have been observed (Christiansen, pers. comm.) and Jena and Patnaik (1990) reported decreased glucose uptake in livers of old lizards.

The present study examines histologic changes in the great arteries of the Yellow Mud Turtle, *Kinosternon flavescens* from Iowa and northern Nebraska. No published work exists on senescence of turtles of the family *Kinosternidae,* and little physiological work on the cardiovascular system of this family has appeared. Most physiological studies have been done with turtles of the family *Emydidae,* especially the red-eared turtle, *Trachemys scripta.* Most of this work supports the idea that reptilian tissues are "heartier" than mammalian tissues. Wright and Hurn (1993) demonstrated that arterial responses of red-eared turtles were insensitive to temperature changes from 15-37 degrees Celcius during *in vitro* experiments. Removal of the endothelium resulted in enhanced responsiveness to norepinephrine and reduced responsiveness to acetylcholine. Acetylcholine initiates contraction of vascular smooth muscle cells in some lower vertebrates (Miller and Vanhoutte 1986, Wright and Hurn 1993), a response opposite from that typically seen in mammals. An explanation for the acetylcholine-induced contraction in turtles may be related to the observation that vertebrate blood vessels follow a phylogenetic

progression from primarily cholinergic innervation in teleosts to adrenergic innervation in mammals (Miller and Vanhoutte, 1986). Unlike mammalian artery, turtle artery exhibits spontaneous contraction during *in vitro* experiments (Miller and Vanhoutte 1986, Wright and Hurn 1993).

Aging in mammals has been well studied. Arteries show increased thickening of arterial wall components with an increase in age (Anderson 1971, Auerbach 1968, Christiansen and Gryzbowski 1993, Cohen 1976, Comfort 1979, Folkow and Svanborg 1993, Pathy 1985). Much of this thickening is the result of the deposition of connective tissue within all layers of the artery. No studies have investigated this phenomenon in reptiles.

Characteristics of the physiological response of senescent reptilian vascular smooth muscle to traditional agonists are unknown. Examination of the vast information on the physiology of aging mammals provides a foundation for study of reptiles. Turtle aortic sections constrict in response to acetylcholine, norepinephrine, and potassium chloride (Miller and Vanhoutte 1986, Wright and Hurn, 1993). Since these substances and their effects have been studied in aging mammals, they were selected as a starting point for physiological studies of aging reptilian vascular smooth muscle.

Senescent mammalian arteries lose their responsiveness to norepinephrine (Cohen and Berkowitz, 1976) although this is thought to be due to intracellular mechanisms and not to a decrease in the number of receptors or decreased binding ability of norepinephrine. Because emydid turtle arteries constrict in response to norepinephrine (Miller and Vanhoutte 1986, Wright and Hurn, 1993), this compound was tested on kinosternid turtles in the present study to determine if they respond in a similar manner and if this response is influenced by age. An elevated potassium ion concentration causes a strong contraction in mammalian artery and its effects are decreased with age (Cohen and Berkowitz 1976, Folkow and Svanborg 1993). This, too, produces a response in emydid turtles and was

tested to determine if it produces a similar response in the kinosternid and if this response is influenced by age. Isoproterenol was selected to determine whether beta-induced relaxation responses decrease in strength due to age as they do in mammals (Folkow and Svanborg 1993).

Isoproterenol (a beta adrenergic agonist) produces relaxation in mammalian vascular smooth muscle cells, a response that declines with age (Cohen and Berkowitz 1976). The vascular response to isoproterenol appears not to have been tested on reptilian systems. The present study examines the effects of this compound on the arteries of old and young kinosternid turtles. If isoproterenol were to produce relaxation of reptilian vascular smooth muscle cells, then it would suggest that vascular control operates through mechanisms similar to mammals.

A significant aspect of this study is that it focuses on a family of turtles (*Kinosternidae*) which is evolutionarily divergent from the turtle family *Emydidae* on which Miller and Vanhoutte (1986) and Wright and Hurn (1993) performed vascular studies. This is the first study of reptilian vascular smooth muscle using tissues of a wild population of known age and it is the first examining tissues histologically and physiologically from the same vessels.

Review of physiology of mammalian vascular smooth muscle

The following review is provided as background for studies of reptilian vascular smooth muscle from Grover and Daniel (1985). In mammals, potassium chloride causes vascular smooth muscle cells to contract by way of voltage-operated calcium channels (VOCCs). The potassium chloride depolarizes the sarcolemma causing the calcium pathways to open. Interstitial calcium ions flood into the sarcoplasm where they bind to calcium receptors on the sarcoplsmic recticulum. This stimulates the release of the calcium ions sequestered by the sarcoplasmic recticulum into the sarcoplasm. Once the cytosolic concentration of calcium reaches 10uM, contraction begins.

Norepinephrine binds with alpha receptors initiating a contractile response, also observed in emydid turtles (Wright and Hurn, 1993). In the present study, phenylephrine was substituted for norepinephrine because it binds specifically to alpha-1 receptors thereby specifying a particular receptor. This was necessary because norepinephrine binds to alpha receptors on vascular smooth muscle cells causing contraction and beta receptors causing relaxation thus making it difficult to determine which receptor was affected by age. When phenylephrine binds to the alpha-1 receptor, contraction occurs by way of two different pathways. The first causes the release of calcium ions from the sarcoplasmic recticulum. This occurs as a result of the alpha receptor activating the inositol lipid second messenger system and promoting the synthesis of inositol triphosphate (IP3). A receptor on the sarcoplasmic recticulum causes the release of sequestered calcium ions when IP3 binds to it. Contraction occurs in the manner mentioned previously. Alpha-1 receptors can also initiate contraction by activating receptor-operated calcium channels (ROCCs) in the sarcolemma. These open a pathway for calcium ions to pass into the cell. Once the intracellular calcium concentration reaches 10uM, contraction occurs.

Acetylcholine causes vasodialation in mammals but, paradoxically, vasoconstriction in reptiles. In mammalian vasculature acetylcholine binds to muscarinic receptors found on the endothelial lining of the artery initiating an influx of calcium ions into the endothelial cells. The calcium binds to the protein calmodulin which activates NO synthase which in turn serves as a catalyst for the synthesis of nitric oxide from L-arginine. Nitric oxide is secreted by endothelial cells and enters the vascular smooth muscle cell, increasing the production of cGMP. This second messenger promotes calcium eflux from the vascular smooth muscle cell and the sequestering of intracellular calcium into the sarcoplasmic recticulum, initiating relaxation.

In mammalian systems, isoproterenol causes relaxation of vascular smooth muscle cells by binding to beta-2 receptors located on the sarcolemma. This activates cAMP second

messenger system promoting the conversion of ATP to cAMP. cAMP activates protein kinase A which then phosphorylates the calcium pumps of the sarcoplasmic recticulum which then sequester intracellular calcium. The calcium pumps of the cellular membrane are also phosphorylated and promote calcium efflux from the cell. Both mechanisms serve to decrease the calcium ion concentration so relaxation occurs. It is assumed that isoproterenol will have the same effect in reptilian systems, probably via the same mechanism.

Materials and Methods

Animals

Specimens of *Kinosternon flavescens* were obtained from the Big Sand Mound population in Muscatine Co., Iowa under Scientific Collecting Permit #SC2559501 issued to Dr. James L. Christiansen of Drake University. Several other *Kinosternon flavescens* were generously provided by Dr. John B. Iverson from Cherry and Dundee Co.s, Nebraska (Fig. 1). These turtles were from monitored wild populations and were of ages estimated to range from 6 to 35+ years. The ages were determined by counting the number of plastral annulae. Turtles usually add one annulus each year although the accuracy of ages estimated decreases to ±5 years for 20 year-olds and ±10 years for 30 year-olds because the earliest annulae are often worn away and the annulae laid down by *Kinosternon flavescens* over 20 years old are extremely close (Christiansen, pers. comm., Iverson, 1991). The turtles were kept for a short period after capture in glass aquaria at 23°C ± 3° and fed a diet of fish.

Chemicals

All chemicals and drugs were purchased from Sigma Chemical Co., St. Louis, MO.

Aortic ring preparation

The turtles were cooled in a refrigerator to 4°C on the day of experimentation. The first turtle was sacrificed by cerebrospinal alcohol injection and the artery removed by the shell and soft hearts method leaving the shell and internal organs undamaged for related studies. The tissue from this turtle was unresponsive and was not used in physiological studies and the remaining turtles were decapitated to decrease time of arterial procurement.

JLC Catalog no.	Age yrs.	Location state	Vascular Study	Histology Study
5976	10	Nebraska	X	X
5977	35	Nebraska	X	X
5978	11	Nebraska		X
5979	17	Nebraska	X	X
5980	14	Nebraska	X	X
5981	8	Nebraska	X	X
5995	24	Iowa		X
5996	19	Iowa		X
5997	6	Iowa	X	X
5998	25	Nebraska	X	X
5999	26	Iowa	X	X
6000	20	Iowa	X	X

FIG. 1 Table summarizing turtle catalog numbers, age, origin, and tests that were performed on each specimen. "X"s denote the specimen was used for the respective test.

A ring of the systemic aortic arches approximately 2.0 mm in length was removed. The descending aorta which is normally used for this type of procedure was not selected for this experiment as the artery width was too small for insertion of the L-hooks. The arterial section was placed in cold reptilian PSS with the following mM concentrations: 1.5; $CaCl_2$, 100; NaCl, 4.7; KCl, 25; $NaHCO_3$, 1.1; $MgCl_2$, 1.2; $KHPO_4$, and 5.6 glucose at a pH of 7.6 (Wright and Hurn, 1993). Glucose was not added until the day of the experiment, thereby preventing bacterial growth.

After the arterial section had been placed in cold PSS, the adventitia was carefully removed and the ring was placed on two chromium hooks (Fig. 2). The lower hook was fixed and the upper L-shaped hook was connected by a small chain to a Grass FT-03D force displacement transducer and isometric contractions were recorded in grams of tension by the Strawberry Tree Data-Aquisition System operating through a Macintosh IICi computer. The artery was suspended in a glass tissue chamber containing 6.0 ml of reptilian PSS at a temperature of 25 degrees Celcius. This temperature was selected because it was shown to provide optimal performance in Emydid turtle arterial experimentation by Wright and Hurn (1993). The baseline was calibrated by hanging a 1.0 g weight from the transducer and its removal determined a baseline of zero. An error was made in storing the information prior to experimentation and went unnoticed for two of the turtles tested. A factor of deviation from the original 1.0 g baseline could not be determined and the results from both turtles were removed from the vascular study. The rings were equilibrated for 1.0 hr. at 1.0 g of tension as atmospheric air was bubbled through the PSS solution. The solution was changed every 15 min. during equilibration.

Aortic ring experimentation

After the rings had equilibrated for one hour, each was contracted by the addition of KCl (50mM). The artery was allowed to reach maximun contraction then relaxed by

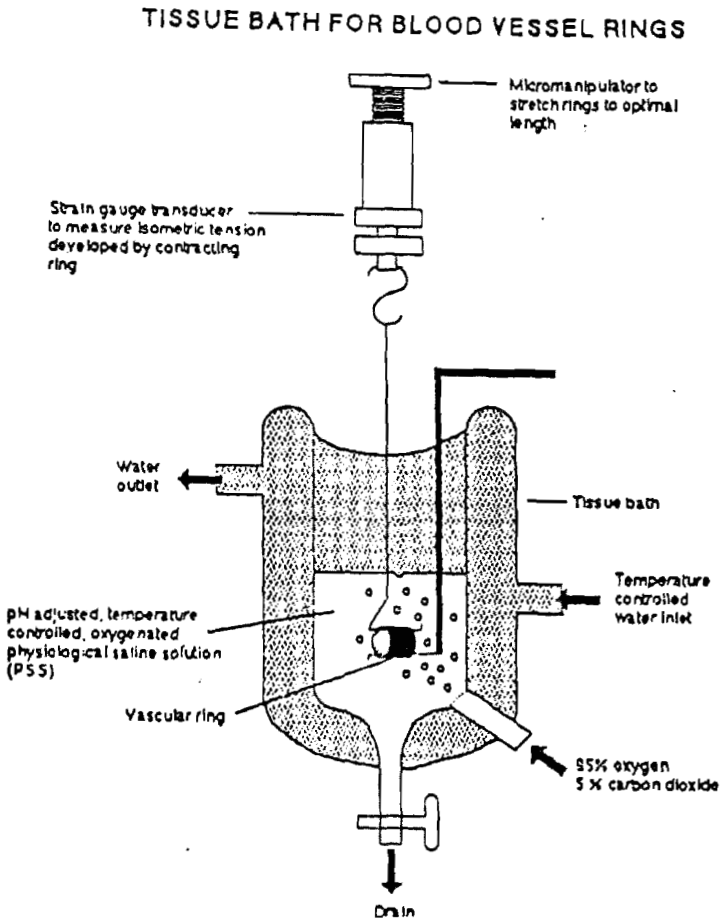

FIG. 2 The apparatus used for studying vascular smooth muscle. The chamber contained 6.0 ml of reptilian PSS and was drained every 15 minutes during equilibration. The temperature of the PSS remained constant as temperature controlled water flowed through the walls of the tissue chamber. (Diagram courtesy of Stratton and Morrow)

several washings of PSS until the tension returned to baseline. PE (10^{-5} M) was then added and after maximun tension was reached, ACh (10^{-5} M) was added to cause further contraction. The artery was returned to baseline tension by repeated washings with PSS. Isoproterenol (10^{-5} M) was added to cause a decrease in tension and the change in tension was recorded. Upon completion of the experimental procedure, the arterial tissue was weighed to the nearest 0.1mg for the purpose of normalizing the tissues. The maximum tension (mg) produced by each neurotransmitter was divided by the weight of the artery and each value was recorded for analysis.

Physiological data analysis

Responses to the chemically induced contractions were analyzed by 2-way ANOVA (analysis of variance). Regression lines were calculated by comparing mg/mg response to turtle age. One plot was analyzed for KCl, PE, ACh, and ISO, respectively. P = 0.05 level of significance was chosen.

Histological preparation

Arterial tissue for histological study was fixed in buffered 10% formalin overnight. The tissue was run through an alcohol dehydration series (45 min; 70%, 45 min; 85%, 45 min; 95%, 45 min; 95%, 45 min; 100%, 45 min; 100%, 45 min; xylene, 45 min; xylene), then placed in molten parrafin for one hour and moved to a second solution of molten paraffin for 12 hrs. The tissues were imbedded in paraffin blocks and cross-sectioned on a manual microtome. The sections were mounted on glass slides with commercial fixative and placed on a heated drying rack for 12 hrs. They were stained with Mallorys Triple Stain in the usual fashion except that they were pretreated for 4 hrs. in a mixture of phosphotungstic and phosphomolybnic acid to insure staining of all connective tissues. Elastin, collagen and other connective tissues take a dark blue stain whereas muscle stains dark pink or red. Coverslips were fixed to the slides with commercial coverslip mounting media and cleaned in preparation for examination.

The first practice runs with arteries from other reptilian species did not provide adequate coloration for the Mallory staining procedure. Connective tissue should stain a deep blue and a red or pink color indicates the presence of muscle. The color we obtained was not suitable for quantifying connective tissue so we searched for a way to increase the perfusion of stain into our specimen. We decided upon soaking mounted, unstained slides in a mixture of phosphomolybnic and phosphotungstic acid for 4 hrs. after paraffin removal by xylene. This produced slides worthy of examination and the acid wash became part of our staining protocol.

Histological examination

Quantification of connective tissue in the tunica media was evaluated in a double-blind procedure. During this procedure all slides had their labels masked and the slides were evaluated without knowledge of the turtles' ages. The slides were only identified by number and after all the results had been recorded, the masked labels were revealed to show the ages of the specimens. Two different individuals quantified connective tissue to insure that the protocol for quantification was appropriate, accurate, and reproducible. All slides were examined under 100X and 400X magnification and quantities of connective tissue were obtained by using an optical micrometer (Fig. 3). The optical micrometer was placed perpendicular to the tunica media and the width of the media was recorded. All areas of blue stain that crossed the line of the micrometer were added and this sum was divided by the tunica media width. The amount of connective tissue was expressed as a percentage of the total width. Three good quality sites on each arterial section were selected at random and the percentages were calculated for each section. The three percentages were averaged to give a final percentage of connective tissue for each specimen.

In cases where the blue color generated by the connective tissue did not entirely fill the graduation on the micrometer scale, we recorded a graduation as full if it was one-half

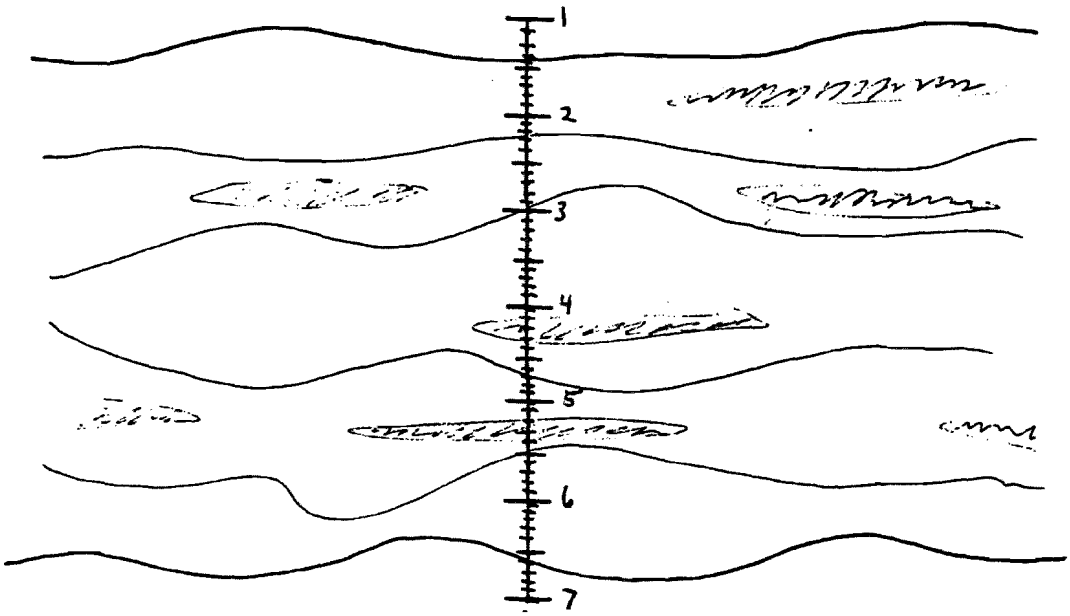

FIG. 3 An optical micrometer used for the quantification of connective tissue. After length of the media was measured, the blue areas of connective tissue were measured and its sum divided by the media length to give a percentage of connective tissue present.

or more filled. If less than one-half of the space was filled, it was not recorded as a full graduation. Evaluation by two different people did produce similar results and this protocol was accepted as our method for connective tissue quantification.

Histological data analysis

The percentages of connective tissue were compared with turtle estimated ages by 2-way ANOVA. Regression lines were calculated to determine the extent of connective tissue related to age. Significance was established if $p < 0.05$.

Results

Histological studies

A total of twelve turtles was examined histologically and nine of these were studied physiologically. A regression line plotted to show possible relationships between estimated age and percentage of the arterial wall that is connective tissue is shown in Fig. 4. It is evident from this illustration that a tendency exists for the percentage of connective tissue to increase with turtle age and this relationship is significant (P = 0.031). It is also evident, however, that some individuals accumulate large amounts of connective tissue by "middle age" (approximately 20 years old) and others, less than normal. These variations may reflect the genetic or physiological history of each turtle. They certainly reflect the fact that age estimates are often inaccurate by the time a turtle has accumulated 20 observable plastoral annulae. Other studies have indicated an accuracy of ±5 years for turtles estimated to be 20 years old and ±10 years for turtles estimated to be 30 years old (Christiansen, pers. comm.). Photomicrographs of sectioned arteries of 6, 20, and 30 year old turtles (Fig. 5) show arteries typical for mud turtles in these age groups. The increasing amount of blue staining reflects increases in the connective tissue. It appears, too, that as connective tissue increases in the oldest turtles, total muscle decreases although this has not been measured quantitatively

Physiological studies

The addition of KCl produced an average response of 0.13 ± 0.06g by living suspended arterial rings (Fig. 6). No statistically significant relationship between age and strength of contraction was observed (P = 0.1364). Phenylephrine produced an average aortic response of 0.07 ± 0.06g. There was no relationship suggested for the impact of phenylephrine on arterial tissue of turtles of different ages (Fig. 7), P = 0.3695. Average tension produced by addition of acetylcholine was 0.29 ± 0.08g. No suggested

relationship is found between acetylcholine response and turtle age (Fig. 8), P = 0.8890. As mentioned earlier, the physiological mechanism for acetylcholine response in reptiles has not been described. Isoproterenol responses were too minimal to conclusively determine whether relaxation had occurred. There is no suggested relationship between isoproterenol response and age (Fig. 9), P = 0.4510. In general, the experiments collectively imply that there is no loss in responsiveness with age even though senescent changes are clearly evident histologically.

ANOVA Table
AGE, yrs vs. %Conn. Tissue

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	314.446	314.446	6.258	.0314
Residual	10	502.471	50.247		
Total	11	816.917			

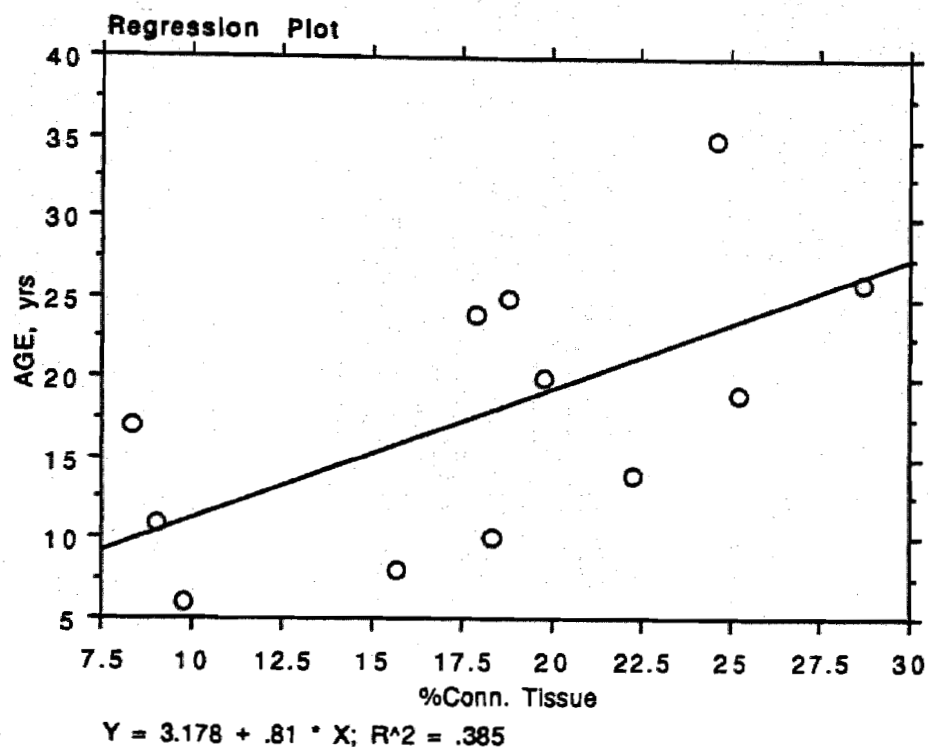

FIG. 4 Regression plot of estimated age of *Kinosternon flavescens* and percentage of arterial wall composed of connective tissue. (P = 0.0314)

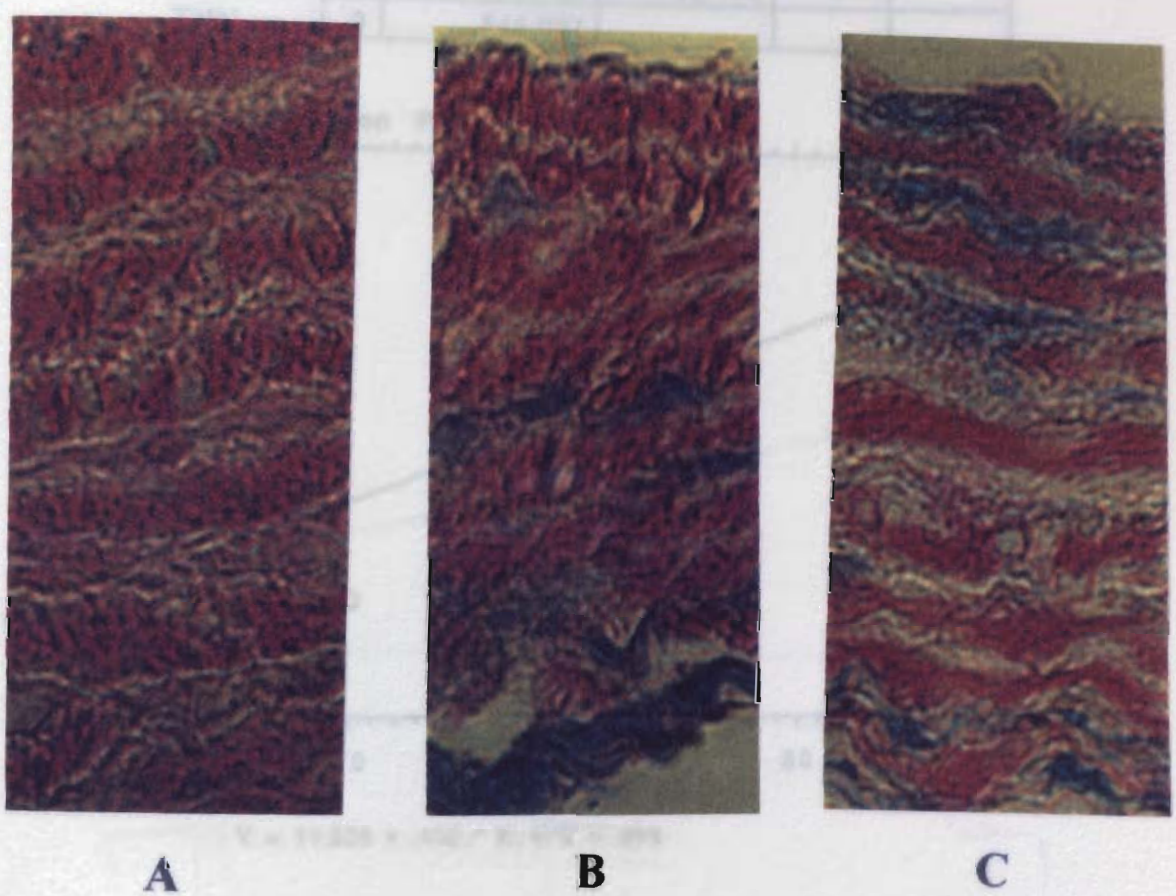

FIG. 5 Sectioned arterial wall of *Kinosternon flavescens*, 1150X. A = 6 year old, B = 20 year old, C = 35+ year old. All ages are estimates based on a count of plastron annulae.

ANOVA Table
KCl, mg/mg vs. AGE, yrs

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	156.944	156.944	2.830	.1364
Residual	7	388.153	55.450		
Total	8	545.097			

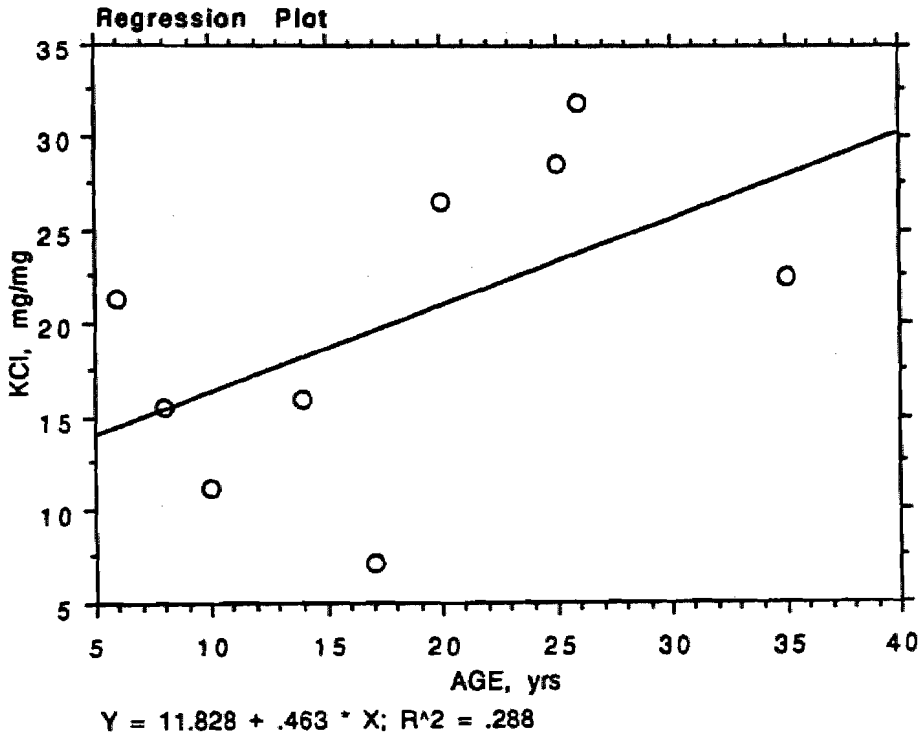

FIG. 6 Regression plot of estimated age of *Kinosternon flavescens* and mg/mg response of arterial tissue by KCl. (P = 0.1364)

ANOVA Table
PE1, mg/mg vs. AGE, yrs

	DF	Sum of Squares	Mean Square	*F*-Value	P-Value
Regression	1	83.718	83.718	.920	.3695
Residual	7	637.318	91.045		
Total	8	721.036			

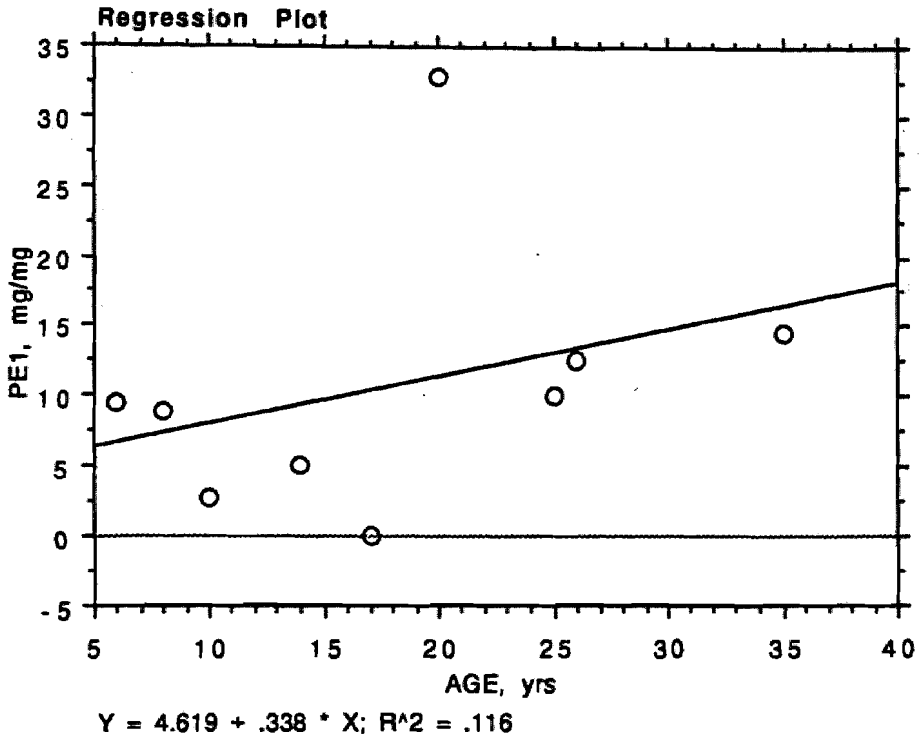

FIG. 7 Regression plot of estimated age of *K. flavescens* and mg/mg response to stimulation by phenylephrine. (P = 0.3695)

ANOVA Table
ACh, mg/mg vs. AGE, yrs

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	7.118	7.118	.021	.8892
Residual	7	2385.802	340.829		
Total	8	2392.920			

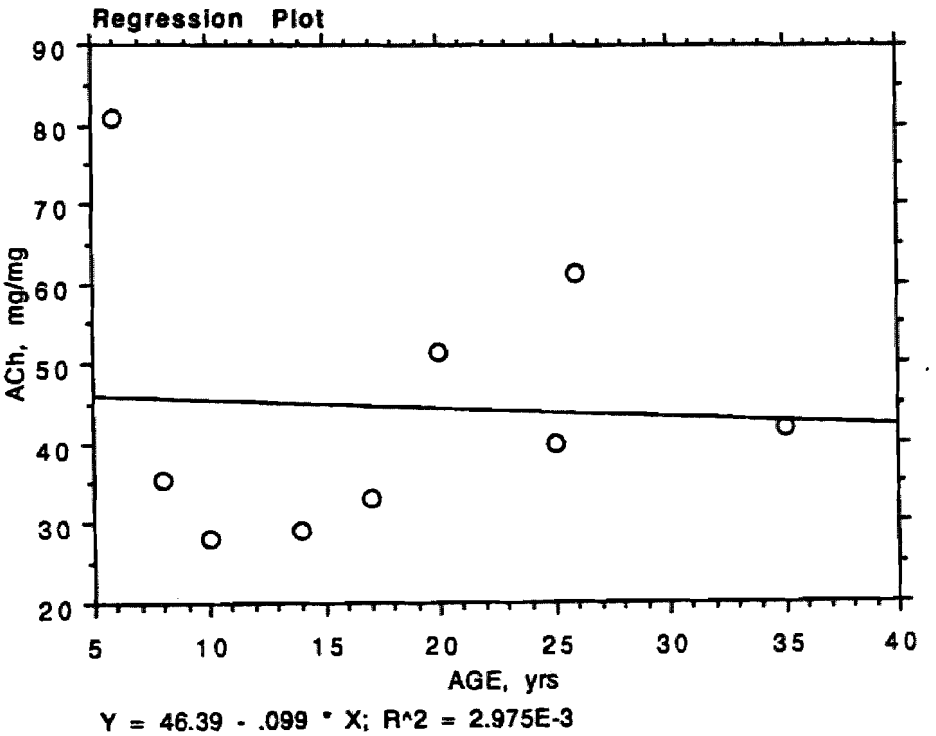

FIG. 8 Regression plot of estimated age of *K. flavescens* and mg/mg response to stimulation by acetylcholine. (P = 0.8892)

ANOVA Table
ISO, mg/mg vs. AGE, yrs

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	129.675	129.675	.638	.4505
Residual	7	1421.712	203.102		
Total	8	1551.387			

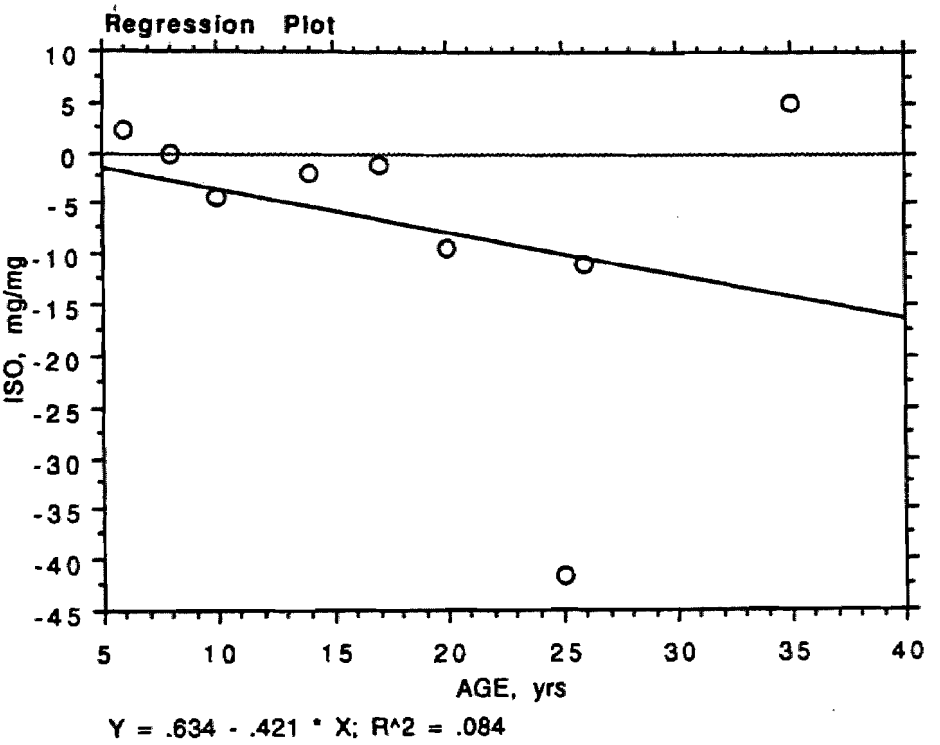

FIG. 9 Regression plot of estimated age of *K. flavescens* and mg/mg response to stimulation by isoproterenol. (P = 0.4505)

Discussion

Decreases in senescent vascular smooth muscle responses in mammals are not the results of thickening artery but possibly due to intracellular mechanisms (Folkow and Svanborg, 1993). The presence of increasing connective tissue and lack of decrease in responsiveness with age in mud turtles suggests senescent connective tissue depostion does not impair agonist-induced constriction. This is consistent with findings in mammals. The response figures (Figs. 6-9) show a general tendency for an increase in response with age though this is not statistically significant. What was surprising was the oldest turtle (35+ years) showed a noticeable degeneration of muscle tissue yet the strength of response did not fall. This could suggest the presence of an age-related compensatory mechanism to account for the absence of a decrease in response. It is possible this mechanism may fail with continued senescence and experimentation on still older turtles may reveal a point at which this occurs. Another explanation may be that the addition of the connective tissue aligns the actin and myosin filaments in a parallel fashion increasing force of contraction.

Responses by isoproterenol on *Kinosternon flavescens* vascular smooth muscle were too small to conclusively state relaxation had occurred. This suggests these turtles do not have beta-2 receptors or they do not operate similarly to mammals. This appears logical as vertebrate blood vessels follow a phylogenetic progression from primarily cholinergic innervation in teleosts to adrenergic innervation in mammals mentioned previously. Turtles demonstrate the presence of alpha adrenergic receptors but beta adrenergic receptors may not have evolved, revealing part of the evolutionary history of beta adrenergic receptors.

The mean tension responses of *Kinosternon flavescens* were much less than responses recorded from vascular studies of *Trachemys scripta.* Wright and Hurn recorded responses of 2.7 ± 0.3g by KCl (50 mM), 3.94 ± 0.27g by ACh (10^{-5} M), and 3.67 ± 0.41g by NE (10^{-5} M) compared to 0.13 ± 0.06g by KCl (50 mM), 0.29 ± 0.08g by ACh

(10^{-5} M) and 0.07 ± 0.06g by PE (10^{-5} M) recorded in the present study. One explanation for the decreased force produced by *Kinosternon flavescens* is that atmospheric air was bubbled through the PSS. Previous studies (Miller and Vanhoutte 1986, Wright and Hurn 1993) used a mixture of 95%O_2 and 5%CO_2 compared to 21%O_2 and 0.4%CO_2 in the atmosphere. The reduced CO_2 would produce less carbonic acid allowing the pH of the PSS to rise. This change in pH may have affected the health of the tissues. Another explanation is that Wright and Hurn (1993), used 2.0 kg *Trachemys scripta* as opposed to the approximately 0.5 kg *Kinosternon flavescens* used in this study. The increased artery mass of the larger turtle would produce more force than the artery from the smaller turtle.

As mentioned previously, the *Kinosternon flavescens* that were obtained were from a wild population that had been monitored so ages of the turtles could be estimated with some accuracy. A problem with selecting *Kinosternon flavescens* as test subjects is that they are a protected species and only a few turtles may be sacrificed for scientific testing. The remains of all test subjects were preserved for the purpose of organ and tissue examination for related research. Turtles obtained from commercial sources would have been unsatisfactory because of the unknown impact of artificial living conditions and ages that could not be estimated with any accuracy. It was believed for the purpose of this study, a wild population of turtles would provide the most accurate results of changes due to aging because the turtles have been relatively free of human influence. Further experimentation would be facilitated if wild populations of turtles of different families were available where ages could be accurately determined and sacrifice of animals is not restricted.

Placing the arterial section on the "L" hook in the tissue bath was difficult because *K. flavescens* is a small species of turtle with an arterial diameter comparable to that of a mouse. The small size increased the time required to get the tissue to an oxygenated environment but this did not seem to be a problem. Fortunately, reptilian tissues are

"heartier" than mammalian tissues (Wright and Hurn, 1993) which is probably why this delay was not observed to be detrimental.

During our experiments, one arterial section from one test specimen was hung in one tissue chamber. Typically, arterial ring hanging experiments are performed using several chambers to hang several sections of artery from the same test animal. The results from all the chambers are averaged to present a more accurate result. We could not use several tissue chambers for our study due to the lack of length of the arteries obtained from our test animals. As mentioned previously, the diameter of the arteries from *K. flavescens* are very small and very short. Several sections could not be obtained for multiple tissue cell experiments. A larger reptile would eliminate this problem.

Conclusions

The yellow mud turtle, *Kinosternon flavescens*, shows a distinct senescent process by the increasing deposition of connective tissue with age. The histological studies show significant depostion of connective tissue throughout the tunica media of the arteries. It also appears that total muscle mass may decline with age. In spite of this age-related change, the turtles maintained or perhaps even increased their strength of vascular smooth muscle response. *Kinosternon flavescens* produced an average vascular smooth muscle response of 0.13 ± 0.06g to KCl (50mM), 0.07 ± 0.06g to PE (10^{-5} M), 0.29 ± 0.08g to ACh (10^{-5} M). ISO produced minimal responses too small to state its effect on *Kinosternon flavescens* artery suggesting beta-2 adrenergic receptors are not present or they do not operate similarly to those of mammals. The aortic responses did not change with age. The lack of changes in pharmacologic responses of vascular smooth muscle in relation to age may suggest the presence of a compensatory mechanism that is initiated to extend life in old turtles and increase the likelihood that females will produce one successful nest.

This study is based on a family of turtles that has not been represented in studies of vascular smooth muscle. It shows that vascular responses of *Kinosternon flavescens* are consistent with the responses of the red-eared Emydid turtle, *Trachemys scripta*, even though the two families are evolutionarily divergent. The turtles in this study were from a natural population and offer a realistic picture of the aging process relatively free from the influences of humans. To our knowledge, no other histological or physiological studies of the impact of age on turtles exist for either captive or wild populations.

This work provides clear evidence of arterial senescence in turtles. Little has been done to study the histopathology of aging in reptiles or physiological changes associated with age. Because these animals are primative when compared to mammals, they may be

the best animals to study when investigating the evolution of the senescence process. There is a slight suggestion of a compensatory mechanism maintaining vascular smooth muscle response in spite of muscle degeneration associated with age. Further studies are needed to evaluate this and to verify the lack of adrenergic beta-2 receptors in turtles.

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